Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 54 (1): 41–45 (2017)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Organic Acid and Nutrient Composition of Lactic Acid Bacteria Inoculated Total Mixed Ration Silage under Tropical Condition

Ahmad Wahyudi[1*], Dicky Pamungkas[2], Roy Hendroko Setyobudi[3, 4], Listriari Hendraningsih[1], and Zane Vincēviča-Gaile[5]

[1]Faculty of Agricultural and Animal Science, Muhammadiyah Malang University, Malang 65144, Indonesia
[2]Institute of Beef Cattle Research, Jl. Pahlawan, Grati, Pasuruan 67184, Indonesia
[3]Ma Chung Research Center for Photosynthetic Pigments, Vila Puncak Tidar N-01, Malang 65151, Indonesia
[4]Indonesia Association of Bioenergy Scientist and Technologist, BPPT Building II, 22nd Floor, Jl. MH. Thamrin No. 8 Jakarta 10340
[5]Department of Environmental Science, University of Latvia, Raina Blvd. 19, Riga, LV-1586, Latvia, European Union

Abstract: Organic acids and nutrients composition of total mixed ration (TMR) silage inoculated with local Lactic acid bacteria (LAB) derived from paddy rice was studied. This study was conducted to prepare TMR silage in tropical climate. LAB was isolated from local paddy rice that was planted around Muhammadiyah University of Malang, East Java, Indonesia. The local LAB ability was compared with commercial strain *L. plantarum* FCC 123 for organic acids and nutrients composition on TMR silage fermentation. The study was designed by completely randomized with five treatments (T0: TMR silage without LAB, T1: TMR silage + *L. plantarum* FCC 123, T2: TMR silage + local LAB Ciherang 1, T3: TMR silage + local LAB Membramo 1, T4: TMR silage + local LAB Rajalele 1), each treatment was replicated four times. This study showed the inoculation of local LAB in TMR silages preparation significantly ($P \leq 0.01$) increased lactic acids production and tended to reduce acetic acid and pH value compare to control. There were not different all treatment and control on nutrients composition. It could be concluded that the local LAB inoculant had ability similar to *L. plantarum* FCC 123 on improving quality of TMR silage.

Keywords: Lactic acid bacteria, nutrient composition, organic acid, silage, total mixed ration

1. INTRODUCTION

Silage is a chopped, fresh plant material that has been preserved by a process of anaerobic fermentation (ensiling) in which organic acids are formed, particularly lactic acid. The ensilage technique was consistently applied until now, even in a number of countries have developed to generate as a functional feed, for supporting livestock productivity and health. The ensilage technique has proven to ensure availability of feed in hard season (winter and long dry season) without reduction of nutrients value. Ensilage changes carbohydrates into lactic acid by lactic acids bacteria (LAB) resulted in a decline of pH to protect the growth of any dangerous microorganisms. The low pH silage can be stored for a long time without any decay.

Development technique on ensilage continued until now, even not only use of forage but also to availability of raw materials including food and beverage industrial waste for example dregs of beer, potatoes and sweet potatoes by-product, molasses, waste tea, palm oil by-product, etc. That needs different methods for making silage. Ensilage technique even has developed on complete feed or total mixed ration (TMR silage). Based on consideration of economic efficiency some improvement methods of ensilage have been done in beef cattle fattening and dairy industry. Some new methods of ensilage preparation have been studied to increase quality of silage. Currently, silage

Received, January 2017; Accepted, March 2017
*Corresponding author: Ahmad Wahyudi; Email: wahyudi_biotek@yahoo.co.id

preparation is not only done in cold temperate regions but also begins to develop in tropics area. Conserving feed as silage is a strategy to alleviate feed constraints and maintain animal productivity during dry spells in the tropics [1]; however the TMR ensilage technique is not yet commonly applied. In this study, organic acids and nutrients composition would be determined in order to understand effect of local LAB inoculants in TMR silage preparation under tropical condition.

Trend the use of industrial waste as a fodder in tropics like; palm oil by-product, copra by-product, rice bran, rice straw, *tumpi* corn, molasses, shoots of sugar cane, coffee by product, shell of a nut, and another industrial waste is increased. It needs TMR formulation to prepare nutritious feed to cater the needs of cattle for 24 h. This is a real challenge in Indonesia constraining productivity of ruminants. Besides TMR formulation, preservation methods also need to consider of feed availability, quality, and its security. In addition [2] stated that TMR silage has a better aerobic stability in comparison with ensiled feedstuff alone.

Sugar and water contents of silage are important determinants in attaining good quality silage. The raw material of silage must contain more than 2 % of sugar (glucose, sucrose, or fructose) and 35 % to 40 % dry matter [3]. If the sugar content less than 0.5 %, addition of molasses or glucose is necessary. The addition of a cellulose enzyme to produce sugar from crude fiber is also an effective technique in process of silage making [4]. Crude fiber will hydrolysis into glucose as energy source of LAB and will be changed into lactic acid. If the moisture content more than 60 %, silage materials should be dried to reduce of excessive butyric acid and increases lactic acid production, in contrast if the moisture content less than 20 %, the moisture needs to be increased [5].

2. MATERIALS AND METHODS

2.1 TMR Silage Preparation

TMR silages were prepared in a small-scale system of silage fermentation 1 [6]. Ingredients composition of TMR were: 16 % rice straw, 20 % rice bran, 34 % cassava chip, 6 % molasses, 17.5 % soybean meal, 4.5 % fish meal, and 3 % minerals. The ingredients composition was arranged based on contents 14 % of crude protein (CP), 65 % to 70 % total digestible nutrients (TDN), and metabolism energy (ME) 2000 kcal. The TMR were treated with 1 % of inoculants *L. plantarum* FCC 123, local LAB *Ciherang* 1, local LAB *Membramo* 1, local LAB *Rajalele* 1 respectively and un-treatment control. *Ciherang*, *Membramo*, and *Rajalele* were varieties name of local paddy rice respectively. LAB was isolated from local that paddy rice varieties, respectively [6, 7]. Approximately 100 g portion of TMR materials were adjusted at 55 % moisture and packed into plastic film bags (KRIS BR 2205 type, 22 cm × 500 cm) and then the bags sealed with a vacuum sealer machine (KRIS VS200). The bag silos were stored in room temperature (average 25 $^{\circ}$C) for 30 d of incubation.

2.2 Organic Acids Analysis

Fermentation products of TMR silages were determined from cold-water extracts. Wet material (10 g) was homogenized with 90 mL of sterile distillated water [8, 7]. The pH was measured with a glass electrode pH meter (Echem E-512 ex GR Scientific) the organic acid contents were measured by gas chromatography (GC) according to the methods describes by reference [9]. The analytical condition was as follows: cold-water extracts (1 µL) was injected into column RTX-5, 30 mm × 0.25 mm, injector temperatute was 220 $^{\circ}$C, split 70 Kpa and flame ionize detector (FID) temperature was 250 $^{\circ}$C, and retention time 40 min.

2.3 Nutrients Analysis

Samples were dried in forced-air oven at 65 $^{\circ}$C for 48 h and ground to pass a 1 mm screen with a Willey mill (ZM200, Retsch GmbH & Co.). Contents of DM, OM, CP, and EE were analyzed according to methods 934.01, 942.05, 976.05, and 920.39 respectively, of AOAC by reference [10].

2.4 Research Design

The study was designed by one way completely randomized design (CRD) with five treatments (N0: TMR silage without LAB, LP: TMR silage + *L. plantarum* FCC 123, CH: TMR silage + local LAB *Ciherang* 1, MR: TMR silage + local LAB *Membramo* 1, RL: TMR silage + local LAB *Rajalele* 1), each treatment replicate four times. Organic acid contents and nutrients composition were measured as experiment parameters.

Table 1. Effect of tropical inoculants on organic acids composition[+].

Inoculant[*)]	Organic Acids (g kg^{-1})			
	Lactic acid[**)]	Acetic acid[**)]	Propionic acid	Butyric acid
N0	40.87±0.78[a]	29.90 ± 0.46 [a]	nd	nd
LP	94.81±0.78[b]	17.82 ± 0,41 [b]	nd	nd
CH	113.91±0.29[b]	25.07 ± 0.45 [a]	nd	nd
MR	91.30±0,52[b]	17.88 ± 0.13 [b]	nd	nd
RL	113.23±0.44[b]	16.95 ± 0.18 [b]	nd	nd

[*)] N0: no-LAB inoculant, LP: *L. plantarum* FCC 123, CH: *Ciherang* LAB1, MR: *Membramo* LAB1, RL: *Rajalele* LAB1,
[**)] [ab]mean ± SD value on the same column show the significant difference.
[+)] value are mean of four samples.

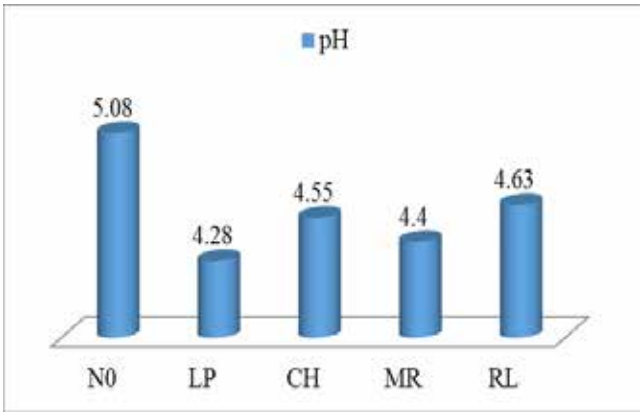

Fig. 1. pH values.

3. RESULTS AND DISCUSSION

3.1 Organic Acids Composition

LAB inoculants gave highly significant ($P \leq 0.01$) effect on increasing lactic acids as described by reference [11, 12]. The lowest lactic acid content was observed in the TMR prepared without inoculants (Table 1). In contras, LAB additions tend to depress acetic acids concentration except for CH that has non significant value with the N0, acetic acid is one of indicator of less than desirable silage fermentation [13], eventhough Driehuis et al. [14] stated that a concentration of acetic acid that ranges from (36 to 50) g kg^{-1} DM is suitable to control yeasts during aerobic exposure of silage. Acetic acid in the products was 16.95 to 29.90 g kg^{-1} DM; Nkosi et al. [15] reported that *L. buchneri* and *P. acidilactici* in TMR silage, after 56 d of ensiling, also play an important role in the aerobic stability of TMR silages.

In this study, there were no significant difference of propionic and butyric acid in all treatments. Propionic acids and butyric acids content were not detected (nd) in all treatments as describe in Table 1, It mean that all TMR silages have prepared in a good condition.

The provision of LAB inoculants showed depress acetic acid production, this may be due to the inoculants type has been used is LAB homo-fermentative where almost 90 % of fermentation product is lactic acid.

The pH value of TMR silage with inoculants were lower than control and ranged pH 4.28 to pH 4.63. This result was in accordance with (16) study for TMR silage. A rather unexpected finding from this study that the pH value was not correlated with lactic acid productions as found in earlier studies. In some case this tendency also found in (17) and (16).

Table 2. Effect of tropical inoculants on nutrients composition[+].

Inoculant[*)]	Nutrient Contents (%)												Energy (kcal)[**)]
	Moisture[**)]		Ash[**)]		OM[**)]		CP[**)]		EE[**)]		CF		NFE[**)]	
N0	56.66 ± 2.3		17.12 ± 2.0		82.88 ± 2.0		14.74 ± 0.5		2.05 ± 0.1		12.35 ± 0.4[***)]		65.57 ± 5.1	2 290.2
LP	57.69 ± 1.0		16.47 ± 3.3		83.53 ± 3.3		14.96 ± 1.0		1.82 ± 0.4		14.91 ± 3.9[***)]		63.84 ± 6.8	2 173.5
CH	54.07 ± 2.3		15.37 ± 1.0		84.64 ± 1.0		14.23 ± 0.7		2.13 ± 0.2		14.18 ± 2.0[***)]		64.55 ± 6.2	2 238.2
MR	53.47 ± 4.4		16.29 ± 1.3		83.71 ± 1.3		15.40 ± 1.7		1.94 ± 0.5		14.06 ± 3.5[***)]		64.33 ± 6.3	2 240.9
RL	53.13 ± 1.2		16.84 ± 3.2		80.16 ± 3.2		15.45 ± 1.5		2.04 ± 0.8		14.35 ± 3.0[***)]		64.53 ± 10.5	2 027.9

[*)] N0: no-LAB inoculant, LP: *L. plantarum* FCC 123, CH: Ciherang LAB1, MR: Membramo LAB1, RL: Rajalele LAB1, OM: organic matter, CP: crude protein, EE; extract ether, CF: crude Fiber, NFE: nitrogen free extract.
[**)] not significant.
[***)] mean ± SD value in the same column show the significant different.
[+)] value are mean of four samples

Acetic acids content 1.5 % to 3.0 % of dry matter could reduce growth of fungi when silage was opened and given to livestock. The acetic acids concentration in this study tended to decline by the addition of LAB inoculants, eventhough *Ciherang* LAB1 yielded relatively same content with no LAB inoculants. Propionic acid on silage could increased if lactic acid would be converted to 1,2-propanediol and further converted to be propionic acid by *L. Diolivorans* [18]. The provision of inoculants in this research has not caused significant differences on propionic acid contents indicate that *L. diolivorans* didn't effect on this TMR silages preparation. On a limited number propionic acid would play a role as acetic acid to reduce growth of fungi at the time when silo were opened and condition became aerobic.

Butyric acid in silage is one indication of contamination clostridia. *Costridia* degrade proteins into butyric acid, caused a decline of protein silage and palatability. *Clostridia* could be prevented by declining pH under 4.5 quickly. Butyric acid contents in this study was not detected on TMR silage with and no LAB inoculants. This condition could be explained that pH was low enough to prevent the growth of *Clostridia* on all treatments, as describe in Table 1 and Fig. 1.

3.2 Nutrients Composition

There were no significant differences on nutrients composition of TMR, both with and without LAB inoculants, except for crude fiber content. Crude fiber decline in TMR without LAB inoculant indicated the silage was fermented by hetero-fermentative microorganisms that has ability to convert some fiber into acetic acid. Generally the nutrients content: moisture, dry matter, organic matter, ash, crude protein, extract ether, nitrogen free extract, and energy were relatively constant. The similar results has been reported by reference [19] that except a little decreasing of water soluble carbohydrate (WSC) and maintain crude fiber, BAL inoculants not affected nutrients composition of silage on mini silo. The results was also strengtened by reference [20] that nutrients composition of silage is not different with or without inoculation of *L. buchneri*. The TMR nutrients composition of this study is given Table 2.

Ensilage could not able to improve the quality of nutrients, but could reduce growth of pathogenic microorganisms (like clostridia), so feed could be preserved for a long period.

4. CONCLUSIONS

This research revealed that local LAB inoculants have the ability similar to the commercially available LAB *L. plantarum* FCC123 inoculants to produce organic acids and preparing the silage with nutrients composition suitable for tropical conditions.

5. ACKNOWLEDGEMENTS

Authors thank to Agung Irawan students for contributions in this research. This publication was part

of a KKP3N Grand Research Funded by Institute for Research and Development, Ministry of Agriculture Republic of Indonesia, No.793/LB.620/l.1/2/2013, Februari 25, 2013.

6. REFERENCES

1. Reiber, C., R. Schultze-Kraft, M. Peters, P. Lentes, & V. Hoffmann. Promotion and adoption of silage technologies in drought-constrained areas of Honduras. *Tropical Grasslands* 44: 231–245 (2010).
2. Nishino, N., H. Harada, & E. Sakaguchi. Evaluation of fermentation and aerobic stability of wet brewers' grains ensiled alone or in combination with various feeds as a total mixed ration. *Journal of Science Food Agriculture* 83: 557–563 (2003).
3. Ohmomo, S., O. Tanaka, H.K. Kitamoto, & Y. Cai. Silage and microbial performance: Old story but new problems. *Japan Agricultural Research Quartely* 36 (2): 59–71 (2002).
4. Khota, W., S. Pholsen, D. Higgs, & Y. Cai. Natural Lactic acid bacteria population of tropical grasses and their fermentation factor analysis of silage prepared with cellulase and inoculant. *Journal of Dairy Science* 99: 1–14 (2016).
5. Pholsen, S., W. Khota, H. Pang, D. Higgs, & Y. Cai. Characterization and application of lactic acid acteria for tropical silage preparation. *Animal Sciences Journal* 87 (10): 1202–1211 (2016)
6. Cai, Y., Y. Benno, M. Ogawa, S. Ohmomo, S. Kumai, & T. Nakase. Influent of *Lactobacillus* spp. from an inoculant and of *Weisella* and *Leuconostoc* spp. from forage crops on silage fermentation. *Applied and Environmental Microbiology* 64: 2982−2987 (1998).
7. Cai, Y., Y. Benno, M. Ogawa, & S. Kumai. Effect of applying lactic acid bacteria isolated from forage crops on fermentation characteristics and aerobic deterioration of silage. *Journal of Dairy Science* 82: 520−526 (1999).
8. Cai, Y., S. Kumai, M. Ogawa, Y. Benno, & T. Nakase. Characterization and identification of *Pediococcus* spp isolated from forage crops and their aplication for silage preparation. *Applied and Environmental Microbiology* 65: 2901−2906 (1999).
9. Wildan, F. Penetapan kadar asam laktat dalam silase dan cairan rumen dengan gas kromatografi [Determination of lactic acid content on silage and rumen fluid by gas chromatography]. *Prosiding Temu Teknis Nasional Tenaga Fungsional Pertanian*. Balai Penelitian Ternak, Ciawi-Bogor (2005). [in Bahasa Indonesia].
10. William, H., & W.L. George. *Official Method of Analysis.* 18th edn. Association of Official Analytical Chemists, Gaithersburg, Maryland, USA. p. 2408−2417 (2005).
11. Filya, I., G. Ashbell, Y. Hen, & Z.G. Weinberg. The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage. *Animal Feed Science and Technology* 88: 39−46 (2000).
12. Muck, R.E., I. Filya, & F.E. Contreras-Govea. Inoculant effects on alfalfa silage: In vitro gas and volatile fatty acid production. *Journal of Dairy Science* 90: 5115–5125 (2007).
13. Mc Donald, P., A.R. Henderson, & S.J.E. Heron. *The Biochemistry of Silage*. Chalcombe Publication, Marlow (1991).
14. Driehuis, F., S.J.W.H. Oude Elferink, & P.G. Van Wikselaar. Fermentation characteristics and aerobic stability of grass silage inoculated with *Lactobacillus buchneri*, with or without homofermentative lactic acid bacteria. *Grass and Forage Science* 56: 330–343 (2001).
15. Nkosi, B.D., R. Meeske, D. Palic, T. Langa, K-J. Leeuw, & I.B. Groenewald. Effects of ensiling whole crop maize with bacterial inoculants on the fermentation, aerobic stability, and growth performance of lambs. *Animal Feed Science and Technology* 154: 193−203 (2009).
16. Hu, X., W. Hao, H. Wang, T. Ning, M. Zheng, & C. Xu. Fermentation characteristics and lactic acid bacteria succession of total mixed ration silages formulated with peach pomace. *Asian Australasian Journal Animal Sciences* 28(4): 502-510 (2015).
17. Chen, L., G. Guo, X. Yuan, M Shimojo, C. Yu, & T Shao. Effect of applying molasses and propionic acid on fermentation quality and aerobic stability of total mixed ration silage prepared with whole-plant corn in Tibet. *Asian Australasian Journal Animal Sciences* 27(3): 349356 (2014)
18. Krooneman, J., F. Faber, A.C. Alderkamp, S.J. Elferink, F. Driehuis, I. Cleenwerck, et. al. *Lactobacillus Diolivorans* sp. nov., a 1, 2-propanediol- degrading bacterium isolated from aerobically stable maize silage. *International Journal of Systematic and Evolutionary Microbiology* 52(2): 639−646 (2002).
19. Huisden, C.M., A.T. Adesogan, S.C. Kim, & T. Ososanya. Effect of applying molasses or inoculants containing *Homofermentative* or *Heterofermentative* bacteria at two rates on the fermentation and aerobic stability of corn silage. *Journal of Dairy Science* 92: 690–697 (2009).
20. Mari, L.J., R.J. Schmidt, L.G. Nussio, C.M. Hallada, & L. Kung. Short communication: An evaluation of the effectiveness of *Lactobacillus buchneri* 40788 to alter fermentation and improve the aerobic stability of corn silage in farm silos. *Journal of Dairy Science* 92: 1174–1176 (2009).